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PATENT SPECIFICATION

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COMPLETE SPECIFICATION

Pharmaceutical Compositions for Oral or Parenteral Administration comprising Tetracycline Antibiotics

We, SOCIETA PRODOTTI ANTIBIOTICI S.p.A., an Italian Joint Stock Company organised under the laws of Italy, of Via Biella 8, Milan, Italy, do hereby declare the invention, for which we pray that a Patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention is concerned with new pharmaceutical compositions for oral or parenteral administration which minimise ill effect caused by the administration of tetracycline antibiotics.

The term "tetracycline antibiotics" as used herein means any antibiotic of the tetracycline series, such as tetracycline, oxytetracycline, bromtetracycline, the corresponding 6-demethyl derivatives and other therapeutically active derivatives in any therapeutically acceptable form, including salts with penicillin, especially penicillin V. Specific examples of such therapeutically active derivatives include the hydrochloride, phosphate and phenoxy-methylpenicillinate of N-[4'-(β-hydroxyethyl)-diethylene-diamino-1'-methyl]-tetracycline, N-(1-pyrrolidinylmethyl)-tetracycline, 7-chloro-6-demethyl-tetracycline and lysine-methyltetracycline.

As is already well known, a local irritating and necrotising action is caused by soluble tetracycline salts when injected into the human body while, in the case of prolonged administration of tetracyclines, well-known, serious secondary phenomena arise.

The necrotising action on the tissues caused by the injection of soluble salts of tetracyclines, is a well known and demonstrated fact. Indeed, the tetracyclines, when administered by intramuscular injection, undergo a direct hydrolysis, tetracycline base, which is an insoluble product exerting a hystolytic action, thereby being liberated. Thus, an acute local pain, due to the irritation of the tissues, is caused in man by the administration of tetracyclines; such a pain is followed by the noticeable phenomena of infiltration which is a painful induration or inflammatory reaction, and by the alteration of venous vessels and arterioles. As a consequence thereof, small thrombi and haemorrhages are caused and blisters and vesicles arise, until a complete necrosis of the tissues occurs. (see, for example, P. Rentnick, Les accidents provoques par les antibiotiques — Antibiotics et Chemotherapy, *1*, 94/1954).

To weaken the sharpness of the pain, use has already been made of mixtures of tetracycline antibiotics with anaesthetics for injection purposes. However, such means only serve to reduce the sharpness of pain, without preventing the incidence of secondary phenomena which, especially in the course of protracted treatment, sometimes result in a necrosis of the tissue at the point of injection.

It is an object of the present invention to provide new pharmaceutical compositions, for oral or parenteral administration, which contain tetracyclines and which prevent the undesired side effects of tetracyclines, in particular the irritating and necrotising phenomena associated with the parenteral administration of tetracyclines, especially in the case of a prolonged administration or in the case of the use of large doses of tetracyclines, and which also prevent, in the case of oral administration of tetracyclines, the occurrence of hypovitaminoses and the like.

Thus, according to the present invention, there are provided new pharmaceutical compositions for oral or parenteral administration containing lysozyme and at least one tetracycline autibiotic, the ratio of lysozyme to tetracycline being at least 0.1:1.

It is well known that lysozyme is an enzyme which is a basic polypeptide having

a positive charge and that it was discovered by Fleming in 1922. It is present in the blood, tissues and body secretions, as well as in tears and human milk. The highest concentrations of lysozyme are found in the brain and in the suprarenal glands. Lysozyme can also be obtained from egg white by various known methods.

We have carried out a series of experimental and pharmacological tests in order to demonstrate the inhibition of necrosis when using a tetracycline antibiotic, together with lysozyme.

Guinea pigs, which are very well suited for the kind of experiments in question, have been used in the experimental tests. A number of guinea pigs of the same family and having an average weight of 350 g, were divided into groups of 10 animals each and depilated in the abdominal region one day before the treatment. A necrotising dose of tetracycline (30 mg. dissolved in sufficient distilled water to give a final volume of 0.5 cc.) was subcutaneously injected in the abdominal region of the animals of a first group (called "control group"). It did not matter which tetracycline derivative was used as the doses were always measured in amounts corresponding to the amount of tetracycline base contained in the tetracycline compounds.

A lysozyme-tetracycline mixture was administered to a test group of guinea pigs by subcutaneous injection using the same methods and techniques used for the control group of animals.

The amounts of lysozyme were 30 and 50 mg, respectively, mixed with a necrotising dose of tetracycline. In other words, one group of 10 animals was treated with a mixture of 30 mg. of tetracycline and 30 mg. of lysozyme, while a second group was treated with a mixture of 50 mg. of lysozyme and 30 mg. of tetracycline.

For an evaluation of the seriousness of particular lesions caused by the administration of tetracycline only, or of equivalent doses of any of its derivatives, the presence and extent of infiltration, of haemorrhage, of blisters and vesicles and of necrosis, was checked at the point of injection, after 24 hours from the start of treatment and for 20 days. The results obtained are expressed in the manner hereinafter indicated:

Evaluation of the local necrotic action, caused by the tetracycline

Infiltrate	+
Infiltrate + haemorrhage	++
Infiltrate + haemorrhage + blisters or vesicles	+++
Infiltrate + haemorrhage + blisters or vesicles + necrosis	++++

The results obtained are set out in the following Table 1:—

TABLE 1

Number of animals tested	Necrotising dose of tetracycline	Quantity of lysozyme added to tetracycline	Seriousness of necrotic lesions
10	30 mg	----	++++
10	30 mg	30 mg	+
10	30 mg	50 mg	no lesions

From the above series of tests, it can be seen that the necrotic lesions, which are very evident following treatment with tetracycline alone, are minimised, or even prevented, by the presence of lysozyme.

Furthermore, in order to ascertain the possibility of preventing, or by the admixture of lysozyme with tetracycline, the secondary phenomena arising as a consequence of a prolonged administration of tetracyclines alone, we have carried out a further series of tests, again on guinea pigs, to which the

drugs were administered both parenterally and orally.

Guinea pigs, having an average weight of 350 g. and divided into four groups, each of which contained 10 animals, were submitted to a treatment with tetracyclines alone, and with the admixture of tetracycline and lysozyme, according to the following Table 2:—

TABLE 2

No. of animals	Administrative route	Tetracycline	Lysozyme	Days	Secondary phenomena
10	oral	100 mg.	----	15	+++++
10	oral	100 mg.	50 mg.	15	none
10	parenteral	70 mg.	----	15	++++
10	parenteral	70 mg.	50 mg.	15	none

The secondary effects referred to in the above Table 2 are loss of weight, haematologic alterations, loss of hair, alterations of organs and death.

Such secondary effects were evaluated as follows:

loss of weight =	+
loss of weight + haematologic alterations =	++
loss of weight + haematologic alterations + loss of hair =	+++
loss of weight + haematologic alterations + loss of hair + alterations of organs =	++++
loss of weight + haematologic alterations + loss of hair + alterations of organs + death	+++++

As can be seen from the Table 2 the phenomena following a prolonged treatment with tetracyclines, i.e. loss of weight, alterations of the haematologic conditions, loss of hair, alterations of organs, and mortality, can be wholly prevented, or at least minimised by administering lysozyme together with the tetracyclines.

We have also carried out clinical tests in order to ascertain the inhibitory action exerted by the admixture of lysozyme with tetracyclines on pain manifestations, on local phenomena caused by the injection of tetracyclines and on the secondary phenomena caused by a prolonged administration of tetracyclines.

In the course of the above clinical tests, the lysozyme was administered to a large number of patients suffering from different infective diseases, having different degrees of seriousness and localisation (for example, bronchial, or pulmonary), together with injectable tetracycline salts, and the course of the local reaction to the injection of tetracyclines, both alone and in admixture with the lysozyme, was closely observed.

These clinical investigations were carried out to demonstrate the most surprising inhibitive action exerted by the lysozyme in respect of the secondary phenomena arising from a prolonged administration (both orally and parenterally) of tetracycline.

In the course of our clinical investigations, both the local and the general secondary effects, due to the hydrolysis and toxicity of the tetracyclines, were taken into consideration. Thus, our investigations were directed to demonstrate how the admixture of lysozyme with tetracycline results in a decrease or in a prevention of the secondary phenomena and thus, by the improvement in the toleration of the antibiotic, how such

an admixture represents a real advance over the products heretofore known.

For the above purpose, investigations directed to ascertain the protective action exerted by the lysozyme against the secondary phenomena which arises locally at the point wherein the drum is injected, were made.

Four groups of 8 patients each, suffering from different infectious diseases, were used. The treatment used is set out in the following Table 3:—

TABLE 3

Number of patients	Administrative route	Tetracycline	Lysozyme	Days	Secondary effects
8	Intramuscular	250 mg.	0	5	++++
8	Intramuscular	250 mg.	50 mg.	5	none
8	Intravenous	250 mg.	0	5	++++
8	Intravenous	250 mg.	50 mg.	5	none

As is clearly shown by Table 3, the secondary effects of treatment with tetracycline, administered intramuscularly or intravenously, can be significantly reduced or attenuated, when lysozyme is admixed with the tetracycline.

In the course of the above investigations, the following phenomena were taken into consideration and evaluated as follows:

1st.	Presence of an infiltration zone =	+
2nd.	Infiltration + haemorrhage =	++
3rd.	Infiltration + haemorrhage + irritation =	+++
4th.	Infiltration + haemorrhage + irritation + pain =	++++

In order to show that the admixture of lysozyme with tetracycline is also able to prevent the secondary phenomena by which prolonged treatment with the tetracycline is accompanied and complicated, we have carried out a further series of clinical tests on patients suffering from different infectious diseases of an intermediate degree of seriousness. These patients were divided into groups, each containing 8 patients. The treatment was given according to the following schedules:

Oral administration

No. of Patients	Tetracycline	Lysozyme	Days	Secondary effects
8	2 g.	----	10	++++
8	2 g.	500 mg.	10	----

Parenteral administration

No. of Patients	Tetracycline	Lysozyme	Days	Secondary effects
8	250 mg.	----	10	++++
8	250 mg.	50 mg.	10	----

The following secondary effects were controlled as a result of clinical investigations: gastro - intestinal intolerance phenomena (atrophy of mucosae, vomiting, sickness and diarrhoea); allergy phenomena (cutaneous erruptions and eosinophilia); dysvitaminosis manifestations (i.e. vitamin deficiency phenomena) due to alterations of the enteric flora; and super-infections (i.e. infections which sometimes appear after treatment with antibiotics and which are sometimes called bacterial rebound), particularly from *Monilia* and *Candida*.

Such secondary effects were evaluated as follows:

Gastro-intestinal intolerance =	+
Gastro-intestinal intolerance + allergy phenomena =	++
Gastro-intestinal intolerance + allergic phenomena + dysvitaminosis =	+++
Gastro-intestinal intolerance + allergy phenomena + dysvitaminosis + superinfections, particularly from *Monilia* and *Candida* =	++++

The new compositions according to the present invention, when administered orally, are used in the form of conventional capsules, as usually employed for antibiotics, and containing a mixture of the two active components in the desired ratio, while, in the case of intramuscular or intravenous administration, a solution of the tetracycline antibiotic is added to a lysozyme solution, the resulting mixture is lyophilised and powders obtained therefrom. These are dissolved in distilled water immediately before use, the whole procedure being carried out in known manner.

Although the weight ratio of lysozyme: tetracycline preferably used is within the range of 0.5:1- 0.2:1 (owing mainly to economic reasons), higher ratios of, for example 0.75:1, 1:1 and 10:1 can also be quite well used; however, the lower limit cannot be reduced below 0.1:1, since otherwise the beneficial effects due to the lysozyme would disappear.

From the above-described experimental and clinical results, it can be safely inferred that the secondary phenomena, which frequently appear in the course of prolonged tetracycline therapy, can be reduced or even wholly prevented, by the new pharmaceutical compositions according to the present invention.

WHAT WE CLAIM IS:—

1. Pharmaceutical compositions for oral or parenteral administration, comprising lysozyme and at least one tetracycline antibiotic (as hereinbefore defined), the ratio of lysozyme to tetracycline being at least 0.1:1.

2. Pharmaceutical compositions according to claim 1, wherein the ratio of lysozyme to tetracycline is within the range of 0.2:1 to 0.5:1.

3. Pharmaceutical compositions according to claim 1 or 2, wherein the tetracycline antibiotic is any one of those hereinbefore specifically mentioned.

4. Pharmaceutical compositions according to claim 1, substantially as hereinbefore described.

H. A. L. VENNER,
Chartered Patent Agent,
Rugby Chambers,
2, Rugby Street,
London, W.C.1.
Agent for the Applicants.

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